REMARKS

Any fees that may be due in connection with this application throughout its pendency may be charged to Deposit Account No. 50-1213.

Claims 2-14, 16-33 and 35-43 are amended. Claims 2-7, 9-14, 16-22, 24-33, 35-39 and 41 are amended to correct obvious typographical and formatting errors and to produce grammatical clarity. Claim 8 is amended to correct an inadvertent error introduced in the claim by the Preliminary Amendment filed with the application on March 1, 2002. The amendment adds an inadvertently deleted subject matter that was originally present in claim 8. The amendment to claim 23 replaces number 5118 by number 5112. The amendment finds basis in SEQ ID NO. 1, which shows 5112 amino acids. In claim 40, an inadvertently omitted phrase "a method selected from the group consisting of" has been added for grammatical clarity. Claims 42 and 43 are amended to add inadvertently omitted subject matter. The amendment finds basis in claim 8, as originally filed.

The specification is amended to correct obvious typographical, spelling and formatting errors and to produce grammatical clarity. In particular the amendments to page 5, line 19, replaces number "5118" with number —5112—. The amendment finds basis in SEQ ID NO. 1, which shows 5112 amino acids.

No new matter has been added.

Included as an attachment is a marked-up version of the specification paragraphs and claims, per 37 CFR §1.121.

* * *

Entry of this amendment and examination of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & McAULIFFE LLP

By: _____
Stephanie L. Seidman
Registration No. 33,779

Attorney Docket No. 24747-1104US
Address all correspondence to:
Stephanie L. Seidman, Esq.
HELLER EHRMAN WHITE & McAULIFFE LLP
4350 La Jolla Village Drive, 7th Floor
San Diego, California 92122-1246
Telephone: 858 450-8400
Facsimile: 858 587-5360
email:sseidman@HEWM.com

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Glare *et al.*

Serial No.: 10/070,489

Filed: March 1, 2002

For: *NUCLEOTIDE SEQUENCES ENCODING AN INSECTICIDAL PROTEIN COMPLEX FROM SERRATIA*

Confirmation No.: 6955

Art Unit: Unassigned

Examiner: Unassigned

ATTACHMENT TO THE PRELIMINARY AMENDMENT
MARKED UP PARAGRAPHS AND CLAIMS (37 CFR §1.121)

IN THE SPECIFICATION:

Please amend the specification as follows:

Please amend the paragraph on page 1, lines 13-21, as follows:

The disease is highly host specific, only [know] known to infect a single indigenous species of New Zealand scarab larva. The disease appears unique among insects and results not from rapid invasion of the haemocoel, but from a slow colonisation of the gut. The disease has a distinct phenotypic progression, with infected hosts ceasing feeding within 2-5 days of ingesting pathogenic cells. The normally black gut clears around this time (Jackson et al. 1993) and the levels of the major gut digestive enzymes (trypsin and so forth) [decreases] decrease sharply (Jackson, 1995). The clearance of the gut results in a characteristic amber colour of the infected hosts. The larvae may remain in this state for a prolonged period (1-3 months) before bacteria eventually invade the haemocoel, causing rapid death.

Please amend the paragraph on page 2, lines 9-14, as follows:

Another region involved in amber disease encoding was located by Nunez-Valdez and Mahanty (1996). They located a locus, *amb2*, by transposon [mutagensis] mutagenesis and searching a cosmid genomic library. This region

was chromosomally located and was involved in antifeeding in the larvae of *Costelytra zealandica*. However, the current applicant's research has demonstrated that the *amb2* region is located on pADAP remote from the virulence gene and is probably regulatory in function.

Please amend the paragraph beginning on page 4, lines 1-8, as follows:

The invention further relates to an isolated nucleic acid molecule comprising a sequence of SEQ ID NO: 1, nucleotides 1955-18937 of SEQ ID NO: 1 or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which [encode] encodes an insecticidal protein. For example, the at least one further nucleotide sequence may be the nucleotide sequence which codes for the *Bacillus* delta toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins and so forth.

Please amend page 5, line 19, as follows:

The polypeptide may comprise amino acids [32-5118] 32-5112 of SEQ ID NO: 1.

Please amend the paragraph page 7, lines 6-8, as follows:

According to a further aspect the invention provides a method of inducing amber disease or like condition in insects comprising [delivery] delivering to an insect an effective amount of the polypeptide of the invention that has functional insecticidal activity against said insect.

Please amend the paragraphs on page 7, line 19, through page 8, line 3, as follows:

The insecticidal polypeptide may be delivered to the insect orally either as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in [an] a transgenic plant, bacterium, virus or fungus upon which the insect feeds, or by any other suitable method which would be obvious to a person skilled in the art.

According to further aspect, the invention provides a transgenic plant, [bacterium] bacterium, virus or fungus, incorporating in its genome, a nucleic acid molecule of the invention providing the plant, [bacterium] bacterium, virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

Please amend the paragraph on page 9, lines 2-5, as follows:

In a further aspect, the present invention consists in replicable transfer vector suitable for use in preparing a polypeptide of the invention. These vectors may be constructed according to techniques well known in the art, or may be selected from cloning [vecotrs] vectors available in the art.

Please amend the paragraph on page 9, lines 11-13, as follows:

Two major types of [vector] vectors possessing these characteristics are plasmids and bacterial viruses (bacteriophages or phages). Presently preferred vectors include pMOS-Blue, pGem-T and pUC8.

Please amend the paragraph on page 12, lines 6-9, as follows:

Preferably, such a nucleic acid construct is a vector comprising a replication system recognised by the host. For the practice of the present invention, well known compositions and techniques for preparing and using vectors, host cells, introduction of vectors into host cells and so [forth.] forth, are employed, as discussed, *inter alia*, in Sambrook et al (1989).

Please amend the paragraph on page 20, lines 3-7, as follows:

Table 1 lists bacterial isolates and plasmids used in the present invention. Bacteria were grown in LB broth or on LB agar (Sambrook et al. 1989), at [37°] 37°C for *Escherichia coli* and 30°C for *S. entomophila*. Antibiotic concentrations used (μg/ml) for *Serratia* were kanamycin 100, chloramphenicol 90, tetracycline 30 and for *e. coli* strains were kanamycin 50, chloramphenicol 30, tetracycline 15, and ampicillin 100.

Please amend the paragraph on page 23, lines 5-17, as follows:

Previously, Grkovic et al. (1995) have shown [taht] that the pADK-13 mutation can be complemented with the pADAP 11 kb *Hin*dIII fragment (gGLA-

20). However, the pADK-10 mutation was unable to be complemented with pGLA-20. In an attempt to isolate the region that may complement the pADK-10 mutation the previously described pGLA-20 derived, pADK-35 null mutation (Grkovic et al. 1995) was used as a selective marker (Fig 1), to select the *Bgl*II fragment encompassing both the pADK-10 and pADK-35 mutations. pADK-35 DNA was isolated and digested with the restriction enzyme *Bgl*II. The resultant digest was ligated into the *Bam*HI site of bBR322 to form the construct pBG35 (containing 12.8kb *Bgl*II - mini-*Tn10* fragment). pBG35 was placed separately in *trans* with pADK-10 and pGLA-20, and the resultant strains bioassayed against grass grub larvae. Results showed that pBG35 was able to complement the pADK-10 mutant, but was unable to induce any symptoms of amber disease when placed in *trans* with pGLA-20, indicating that there must be another region on pADAP needed to induce amber disease.

Please amend the paragraph on page 26, lines 3-9, as follows:

The large *Bam*HI fragment (18937 bp) derived from the pBM32-8 was sequenced on both strands using a combination of constructed detections, plasmid subclones and custom made primers. A total continuous sequence of 18937 bp has been deposited in Gene Bank (Accession Number AF135182). Structural analysis of the DNA sequence using DNAMAN showed that there was a 12-bp sequence [repealed] <u>repeated</u> five times between positions 683 and 743. The repeat is flanked by an upstream 13 base pair palindrome (669-682-bp), and a degenerate 34-bp downstream palindrome (765-799-bp)(Fig 2d,e).

Please amend the paragraph on page 31, line 18, through page 33, line 4, as follows:

The 23-kb region cloned into pBR322 to make pBM32 conferred pathogenicity in pADAP-cured *S. entomophilia* strains with all symptoms of amber disease being observed. Insertion mutants in pBM32 that abolished pathogenicity were transferred to pADAP. The resultant strains showed a partial disease phenotype, including anti-feeding but not gut clearance, suggesting that an additional anti-feeding gene may be present elsewhere on

pADAP. The occurrence of two different anti-feeding genes on pADAP also supports data of Grkovic et al. (1995) who found that suppression of feeding was stronger in the wild-type pADK-6 strain, compared to the partial disease state (pADK-10, pADK-13) of inducing anti-feeding but no gut clearance. A putative anti-feeding gene, *amb2*, has already been isolated from the genomic DNA of *S. entomophilia* (Nunez-Valdez and Mahanty, 1996). Recent data [indicate] indicates that the *amb2*, locus resides at an as yet to be identified location on pADAP that is remote from the region identified therein (Hurst, unpublished data).

Please amend the paragraph on page 36, lines 10-24, as follows:

Using the polymerase chain reaction (PCR) the initiation [codon] codons ATG of the three *sep* genes (*sepA, sepB* and *sepC*) were individually placed into the unique *Nde*I site (restriction enzyme site (CATGG) of the HIS-tag arabinose expression vector pAV2-10 (obtained from Chuck Shoemaker - AgResearch). Because large proteins i.e. greater than 50 kda are limited in their ability to bind to HIS tag affinity columns the carboxyl terminus of each of the Sep proteins did not need to be in frame with the HIS-tag site. Instead wild type DNA (non PCRd) containing a downstream chloramphenicol resistance gene was ligated into the appropriate restriction enzyme site (*sepA, Sun*I; *sepB Hin*dIII; *sepC Bst*XI) of the pAV2-10-*sep* derived vectors:-

-the use of the chloramphenicol resistant maker provided by the vector pACYC184 enhances the stability to each of the expression constructs i.e. -the antibiotic amphicilin to which the pAV2-10 is resistant too is cleaved in the media to an inactive form leading to possible plasmid free segregants arising. Conversely the antibiotic chloramphenicol is not cleaved heightening the level of plasmid stability under conditions of arabinose induction.

In the Claims:

Please amend claims 2-14, 16-33, 35-43 as follows:

2. (Amended) A purified and isolated nucleic acid molecule as claimed in [Claim 1comprising] claim 1, comprising the nucleotide sequence 1995-18937 of SEQ ID NO: 1.

3. (Amended) A purfied and isolated nucleic acid molecule as claimed in [Claim 1] claim 1, comprising one or more of the nucleotide sequences 2411-9547, 9589-13883 or 14546-17467 of SEQ ID NO: 1.

4. (Amended) A purified and isolated nucleic acid molecule as claimed in [Claim 3] claim 3, comprising all of nucleotide sequences 2411-9547, 9598-13884 and 14546-17467 of SEQ ID NO: 1.

5. (Amended) A purified and isolated nucleic acid molecule as claimed in [Claim 1] claim 1, comprising a sequence of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which [encode] encodes an insecticidal protein.

6. (Amended) A purified and isolated nucleic acid molecule as claimed in [Claim 2] claim 2, comprising nucleotides 1955-18937 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which [encode] encodes an insecticidal protein.

7. (Amended) A purified and isolated nucleic acid molecule as claimed in [Claim 3] claim 3, comprising a sequence of SEQ ID NO: 1,or one or more of nucleotides 2411-9547, 9598-13884 or 14546-17467 of SEQ ID NO: 1, operably linked to at least one further nucleotide sequence which [encode] encodes an insecticidal protein.

8. (Amended Twice) A purified and isolated nucleic acid molecule as claimed in claim 4, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.

9. (Amended) A purified and isolated nucleic acid molecule as claimed in [claim 1] claim 1, wherein nucleic acid molecule may comprise DNA, cDNA or RNA.

10. (Amended) A purified and isolated nucleic acid molecule as claimed in [claim 1] claim 1, wherein the nucleic acid molecules said fragment, neutral mutation or homolog thereof capable of hybridising to said nucleic acid molecule, hybridise to the nucleotide sequence of SEQ ID NO: 1, or nucleotides 1955-18937, 2411-9598-13884 or 14546-17467 of SEQ ID NO: 1 if there is at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity between the sequences.

11. (Amended) A purified and isolated nucleic acid molecule as claimed in [claim 1] claim 1, wherein the nucleic acid molecule may be isolated from *Serratia entomophilia* or *Serratia proteamaculans* strains of bacteria.

12. (Amended) A recombinant expression vector(s) containing the nucleic acid molecule as claimed in [Claim 1] claim 1 and host transformed with the vector expressing a polypeptide.

13. (Amended) A recombinant expression vector(s) as claimed in [claim 11] claim 12, wherein the vector is selectable from any suitable natural or artificial plasmid/vector.

14. (Amended) A recombinant expression vector(s) as claimed in [claim 13] claim 13, wherein said suitable natural or artificial plasmid/vector, including pUC 19 (Yannish-Perron et al. 1995), pProEX HT (GibcoBRL, Gaithersburg, MD, USA), pBR322 (Bolivar et al 1977), pACYC184 (Chang et al. 1978), pLAFR3 (Staskowicz et al. 1987).

16. (Amended) A method of producing a polypeptide of [claim 15] claim 15, comprising the steps of:

(a) culturing a host cell which has been transformed or transfected with said vector as defined above to express the encoded polypeptide or peptide; and

(b) recovering the expressed polypeptide or peptide.

17. (Amended) A ligand that binds to a polypeptide of [Claim 15] claim 15.

18. (Amended) A ligand as claimed in [claim 17] claim 17, wherein the ligand is an antibody or antibody binding fragment.

19. (Amended) Probes and primers comprising a fragment of the nucleic acid molecule as claimed in [Claim 1] claim 1, wherein said fragment is hybridisable under stringent conditions to a native insecticidal gene sequence.

20. (Amended) Probes and primers comprising a fragment of the nucleic acid molecule as claimed in [claim 19] claim 19, wherein said probes and primers enable the structure and function of the gene to be determined and homologs of the gene to be obtained from bacteria other than *Serratia* sp.

21. (Amended) A polypeptide as claimed in [Claim 15] claim 15, wherein the polypeptide has insecticidal activity encoded by the nucleic acid molecule of claim 1, or a functional fragment, neutral mutation or homolog thereof.

22. (Amended) A polypeptide having insecticidal activity as claimed in [claim 21] claim 21, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 or a functional fragment, neutral mutation or homolog thereof.

23. (Amended) A polypeptide having insecticidal activity as claimed in [claim 21] claim 21, wherein the polypeptide comprises amino acids 32-5112 of SEQ ID NO: 1.

24. (Amended) A polypeptide having insecticidal activity as claimed in [claim 21] claim 21, wherein the polypeptide comprises at least one amino acid sequence of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5 or SEQ ID NO: 6.

25. (Amended) A polypeptide having insecticidal activity as claimed in [claim 24] claim 24, wherein the polypeptide preferably comprises amino acid sequence SEQ ID NO: 4; SEQ ID NO: 5 and SEQ ID NO: 6.

26. (Amended) A polypeptide having insecticidal activity as claimed in [claim 24] claim 24, wherein the polypeptide preferably comprises all of SEQ NOs: 2-6.

27. (Amended) A polypeptide having insecticidal activity as claimed in [claim 21] claim 21, wherein the polypeptide is obtained by expression of a DNA sequence coding therefore in a host cell or organism.

28. (Amended) A polypeptide having insecticidal activity as claimed in [claim 27] claim 27, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO: 1 linked to at least one further amino acid sequence encoding an insecticidal protein.

29. (Amended) A polypeptide having insecticidal activity as claimed in [claim 28] claim 28, wherein the at least one further amino acid sequence includes the amino acid sequence which codes for *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

30. (Amended) A polypeptide having insecticidal activity as claimed in [claim 28] claim 28, wherein the polypeptides comprise at least 50%, preferably 60%, more preferably 70% and most preferably 90-95% or greater identity to SEQ ID NO: 1.

31. (Amended) A polypeptide having insecticidal activity as claimed in [claim 21] claim 21, wherein the polypeptide is produced by expression of a vector comprising the nucleic acid of SEQ ID No:1 or a functional fragment, neutral mutation or homolog thereof, in a suitable host cell.

32. (Amended) An insecticidal composition comprising at least the polypeptide as claimed in [claim 21] claim 21, and an agriculturally acceptable carrier.

33. (Amended) An insecticidal composition as claimed in [claim 32] claim 32, wherein more than one polypeptide is included in the composition.

35. (Amended) An insecticidal composition as claimed in [claim 34] claim 34, wherein the composition comprises other known insecticidally active agents, including *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifermentens* mosquitocidal toxins and/or *Photorhabadus luminescens* toxins.

36. (Amended) A method of combating pests, said method comprising applying to a locus, host and/or the pest, an effective amount of the polypeptide as claimed in [Claim 21] claim 21, that has functional insecticidal activity against said pest.

37. (Amended) A method of inducing amber disease or like condition in insects comprising delivery to an insect an effective amount of the polypeptide as claimed in [Claim 21] claim 21, that has functional insecticidal activity against said insect.

38. (Amended) A method of inducing amber disease or like condition in insects as claimed in [claim 37] claim 37, comprising delivery to an insect an effective amount of the polypeptide wherein the insect is selected from the order comprising Coleoptera.

39. (Amended) A method of inducing amber disease or like condition in insects as claimed in [Claim 38] claim 38, comprising delivery to an insect an effective amount of the polypeptide wherein the insect includes *Costelytra zealandica* (Coleoptera: Sacarabaeidae).

40. (Amended) A method of delivering the insecticidal polypeptide to induce amber disease or like condition in insects including delivery of the insecticidal polypeptide as claimed in [Claim 39] claim 39, to the insect by [any one of] a method selected from the group consisting of: presenting the insecticidal polypeptide orally as a solid bait matrix, as a sprayable insecticide sprayed onto a substrate upon which the insect feeds, applied directly to the soil subsurface or as a drench or is expressed in an transgenic plant, bacterium, virus or fungus upon which the insect feeds.

41. (Amended) A transgenic plant, [bacterium] bacterium, virus or fungus, incorporating in its genome, a nucleic acid molecule as claimed in [Claim 1] claim 1, for providing the plant, [bacterium] bacterium, virus or fungus with an ability to express an effective amount of an insecticidal polypeptide.

42. (Amended) A purified and isolated nucleic acid molecule of claim 5, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta

endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.

43. (Amended) A purified and isolated nucleic acid molecule of claim 6, wherein the sequence of nucleotides encodes at least one of the *Bacillus* delta endo toxins, vegatative insecticidal proteins (vips), cholesterol oxidases, *Clostridium bifementens* mosquitocidal toxins or *Photorhabadus luminescens* toxins.